研究成果の刊行に関する一覧表　平成 22 年度(2010)

<雑誌>

研究代表者：斎藤博久、分担研究者：松本健治、大矢幸弘

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Morita H, Arae K, Ohno T, Kajiwara N, Oboki K, Matsuda A, Suto H, Okumura K, Sudo K, Takahashi T, Matsumoto K, Nakae S.	ST2 requires Th2-, but not Th17-, type airway inflammation in epicutaneously antigen- sensitized mice.	Allergol Int		[Epub ahead of print] 2012 Feb 25.	2012
Futamura M, Ohya Y, Akashi M, Adachi Y, Odajima H, Akiyama K, Akasawa A.	Age-related Prevalence of Allergic Diseases in Tokyo Schoolchildren.	Allergol Int	60(4):	509-15.	2011
Noguchi E, Sakamoto H, Hirota T, Ochiai K, Imoto Y, Sakashita M, Kurosaka F, Akasawa A, Yoshihara S, Kanno N, Yamada Y, Shimojo N, Kohno Y, Suzuki Y, Kang MJ, Kwon JW, Hong SJ, Inoue K, Goto Y, Yamashita F, Asada T, Hirose H, Saito I, Fujieda S, Hizawa N, Sakamoto T, Masuko H, Nakamura Y, Nomura I, Tamari M, Arinami T, Yoshida T, Saito H, Matsumoto K.	Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations.	PLoS Genet	7(7):	e1002170.	2011

分担研究者：片山一朗

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kitaba S, Murota H, Terao M, Azukizawa H, Terabe F, Shima Y, Fujimoto M, Tanaka T, Naka T, Kishimoto T, Katayama I	Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma.	Am J Pathol.	180	165-76	2012

Terao M, Murota H, Kimura A, Kato A, Ishikawa A, Igawa K, Miyoshi E, Katayama I	11β-hydroxysteroid dehydrogenase-1 is a novel regulator of skin homeostasis and a candidate target for promoting tissue repair.	PLoS One.	;6(9)	e25039	2011
Murota H, Katayama I	Assessment of antihistamines in the treatment of skin allergies.	Curr Opin Allergy Clin Immunol.	11(5)	428-37	2011
Terao M, Ishikawa A, Nakahara S, Kimura A, Kato A, Moriwaki K, Kamada Y, Murota H, Taniguchi N, Katayama I, Miyoshi E	Enhanced Epithelial-Mesenchymal Transition-like Phenotype in N-Acetylglucosaminyltransferase V Transgenic Mouse Skin Promotes Wound Healing.	J Biol Chem.	286(32)	28303-11	2011
Murakami Y, Wataya-Kaneda M, Terao M, Azukizawa H, Murota H, Nakata Y, Katayama I	Peculiar distribution of tumorous xanthomas in an adult case of erdheim-chester disease complicated by atopic dermatitis.	Case Rep Dermatol.	3(2)	107-12	2011
Murakami Y, Matsui S, Kijima A, Kitaba S, Murota H, Katayama I	Cedar pollen aggravates atopic dermatitis in childhood monozygotic twin patients with allergic rhino conjunctivitis.	Allergol Int.	60(3)	397-400	2011
Kitaba S, Matsui S, Iimuro E, Nishioka M, Kijima A, Umegaki N, Murota H, Katayama I	Four Cases of Atopic Dermatitis Complicated by Sjögren's Syndrome: Link between Dry Skin and Autoimmune Anhidrosis.	Allergol Int.	60(3)	387-91	2011
Murota H, Katayama I	Lichen aureus responding to topical tacrolimus treatment.	Dermatol.	38(8)	823-5	2011
Terao M, Nishida K, Murota H, Katayama I.	Clinical effect of tocoretinate on lichen and macular amyloidosis.	J Dermatol.	38(2)	179-84	2011
Hanafusa T, Tamai K, Umegaki N, Yamaguchi Y, Fukuda	The course of pregnancy and childbirth in three mothers with recessive dystrophic	Clin Exp Dermatol.	37(1)	10-4	2012

S, Nishikawa Y, Yaegashi N, Okuyama R, McGrath JA, Katayama I.	epidermolysis bullosa.				
Hanafusa T, Azukizawa H, Kitaba S, Murota H, Umegaki N, Terao M, Sano S, Nakagiri T, Okumura M, Katayama I	Diminished regulatory T cells in cutaneous lesions of thymoma-associated multi-organ autoimmunity: a newly described paraneoplastic autoimmune disorder with fatal clinical course.	Clin Exp Immunol.	166(2)	164-70	2011
Ogata A, Umegaki N, Katayama I, Kumanogoh A, Tanaka T	Psoriatic arthritis in two patients with an inadequate response to treatment with tocilizumab.	Joint Bone Spine	79(1)	85-7	2012
Nakagawa Y, Takamatsu H, Okuno T, Kang S, Nojima S, Kimura T, Kataoka TR, Ikawa M, Toyofuku T, Katayama I, Kumanogoh A	Identification of semaphorin 4B as a negative regulator of basophil-mediated immune responses.	J Immunol.	186(5)	2881-8	2011
Azukizawa H, Döhler A, Kanazawa N, Nayak A, Lipp M, Malissen B, Autenrieth I, Katayama I, Riemann M, Weih F, Berberich-Siebelt F, Lutz MB	Steady state migratory RelB+ langerin +dermal dendritic cells mediate peripheral induction of antigen-specific CD4+ D25+ Foxp3 +regulatory T cells.	Eur J Immunol.	41(5)	1420-34	2011
Katayama I, Kohno Y, Akiyama K, Ikezawa Z, Kondo N, Tamaki K, Kouro O	Japanese guideline for atopic dermatitis.	Allergo l Int.	60(2)	205-20	2011

Matsui S, Kitaba S, Itoi S, Kijima A, Murota H, Tani M, Katayama I	A case of disseminated DLE complicated by atopic dermatitis and Sjögren's syndrome: link between hypohidrosis and skin manifestations.	Mod Rheumatol.	21(1)	101-5	2011
室田浩之, 北場俊, 片山一朗他	大阪大学関連施設を中心としたアトピー性皮膚炎患者の生活習慣実態調査研究	J Environ Dermatol Cutan Allergol.	5	103-14	2011
田村忠史、室田浩之、片山一朗	オロパタジンによる痒みと表皮内神経線維の伸長の制御	アレルギーと神経ペプチド	7	32-6	2011
片山一朗	アトピー性皮膚炎の診療ガイドライン.	アレルギー免疫	18（10）	10-20	2011

分担研究者：木戸博

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki K, Hiyoshi M, Tada H, Bando M, Ichioka T, Kamemura N and Kido H.	Allergen diagnosis microarray with high-density immobilization capacity using diamond-like carbon-coated chips for profiling allergen-specific IgE and other immunoglobulins.	Analytica Chimiva Acta	706	321-327	2011
Kamemura N, Tada H, Shimojo N, Morita Y, Kohno Y, Ichioka T, Suzuki K, Kubota K, Hiyoshi M and Kido H.	Intrauterine sensitization of allergen-specific IgE analyzed by a highly-sensitive new allergen microarray.	J Allergy Clin Immunol	In press		2012

Ⅴ．研究成果の刊行物・別刷
（主なもの）

Allergology International. ****;**:***-***
DOI: 10.2332/allergolint.11-OA-0379

ORIGINAL ARTICLE

ST2 Requires Th2-, but Not Th17-, Type Airway Inflammation in Epicutaneously Antigen-Sensitized Mice

Hideaki Morita[1,2], Ken Arae[2,3], Tatsukuni Ohno[2], Naoki Kajiwara[4], Keisuke Oboki[2], Akio Matsuda[2], Hajime Suto[4], Ko Okumura[4], Katsuko Sudo[5], Takao Takahashi[1], Kenji Matsumoto[2] and Susumu Nakae[2,4,6,7,8]

ABSTRACT

Background: IL-33 is known to induce Th2-type cytokine production by various types of cells through its receptors, ST2 and IL-1RAcP. Polymorphism in the ST2 and/or IL-33 genes was found in patients with atopic dermatitis and asthma, implying that the IL-33/ST2 pathway is closely associated with susceptibility to these diseases. Exposure to allergens through damaged skin is suspected to be a trigger for allergen sensitization, resulting in development of such allergic disorders as asthma and atopic dermatitis.

Methods: To elucidate the role(s) of the IL-33/ST2 pathway in asthma in individuals who had been epicutaneously sensitized to an antigen, wild-type and ST2$^{-/-}$ mice were epicutaneously sensitized with ovalbumin (OVA) and then were intranasally challenged with OVA. The degree of airway inflammation, the number of leukocytes and the activities of myeloperoxidase (MPO) and eosinophil peroxidase (EPO) in bronchoalveolar lavage fluids (BALFs), The levels of cytokines and chemokines in lungs and OVA-specific IgE levels in sera were determined by histological analysis, a hemocytometer, colorimetric assay, quantitative PCR or ELISA, respectively.

Results: The number of eosinophils in BALFs, the levels of Th2 cytokines and chemoattractants in the lungs and OVA-specific IgE in sera from ST2$^{-/-}$ mice were significantly reduced compared with wild-type mice. Although the number of neutrophils in BALFs and the pulmonary levels of IL-17 were comparable in both mice, the levels of MPO activity in BALFs and neutrophil chemoattractants in the lung were reduced in ST2$^{-/-}$ mice.

Conclusions: The IL-33/ST2 pathway is crucial for Th2-cytokine-mediated eosinophilic, rather than Th17-cytokine-mediated neutrophilic, airway inflammation in mice that had been epicutaneously sensitized with antigens and then challenged with antigen.

KEY WORDS

asthma, eosinophils, epicutaneous sensitization, IL-33, ST2

[1]Department of Pediatrics, Keio University School of Medicine, [2]Department of Allergy and Immunology, National Research Institute for Child Health and Development, [3]Department of Immunology, Faculty of Health Science, Kyorin University, [4]Atopy Research Center, Juntendo University School of Medicine, [5]Animal Research Center, Tokyo Medical University, [6]Frontier Research Initiative, [7]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo and [8]Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama, Japan.

Conflict of interest: No potential conflict of interest was disclosed.
Correspondence: Susumu Nakae, PhD, Frontier Research Initiative, and Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4–6–1 Shirokanedai, Minato, Tokyo 108–8639, Japan.
Email: snakae@ims.u-tokyo.ac.jp
Received 29 September 2011. Accepted for publication 11 October 2011.

INTRODUCTION

Several longitudinal epidemiological studies proved that eczema is the first clinical manifestation of allergic diseases during infancy, followed by the development of atopic asthma and rhinitis.[1-4] The underlying mechanisms of allergic disease development remain unclear, but exposure to allergens through skin damaged by disruption of the epidermal barriers is suspected to be a trigger for sensitization in allergic disorders. In support of this notion, genetic deficiency for *filaggrin*, which is crucial for formation of epidermal barriers, is considered to be a predisposing factor for certain allergic diseases such as atopic dermatitis, asthma and rhinitis.[5-8] Notably, filaggrin-deficient mice develop dermatitis after epicutaneous exposure to protein antigens.[9] In addition, mice that had been epicutaneously sensitized with an allergen develop allergic airway inflammation after inhalation of the same allergen.[10-13] These findings suggest that epicutaneous exposure to antigens due to dysfunctional epidermal barriers contributes strongly to induction of allergic diseases.

IL-33, a member of the IL-1 family of cytokines, is able to induce production of Th2-type cytokines by various types of cells such as Th2 cells and mast cells through its ST2 and IL-1RAcP receptors.[14] IL-33 is localized in cell nuclei[15] and released by necrotic cells after tissue injury,[16-18] suggesting that it may be released by damaged skin after scratching, thereby contributing to the development of allergic disorders. In support of this, IL-33 mRNA/protein levels are elevated in skin lesions of patients with atopic dermatitis.[19] Polymorphism of the ST2 and/or IL-33 genes was found in patients with atopic dermatitis, asthma and rhinitis,[20-26] implying an association with disease severity. However, the role(s) of the IL-33/ST2 pathway in allergic airway inflammation in individuals who had been epicutaneously sensitized to an antigen remains unclear. We attempted to elucidate its role (s) by studies in ST2-deficient mice.

METHODS

MICE

BALB/cA wild-type mice were purchased from Sankyo Lab (Tsukuba, Japan). ST2$^{-/-}$ mice (on the BALB/c background) were generated as described elsewhere.[27] Six- to 9-week-old female mice were used in all experiments. Mice were housed under specific-pathogen-free conditions at the National Research Institute for Child Health and Development, and the animal protocols were approved by the Institutional Review Boards of the National Research Institute for Child Health and Development and The Institute of Medical Science, The University of Tokyo.

EPICUTANEOUS SENSITIZATION

Mice were epicutaneously sensitized with OVA as described elsewhere,[13] with minor modifications. In brief, the dorsal skin of mice was shaved with hair clippers and then stripped 6 times with adhesive cellophane tape (Nichiban, Tokyo, Japan). A patch (Finn chamber disk; ϕ8 mm; Smart Practice, Phoenix, AZ, USA) containing an antigen solution (400 μg of OVA [grade V; Sigma-Aldrich, St. Louis, MO, USA] in 40 μl of PBS) or PBS alone (control) was placed on the tape-stripped skin for 3 days and then removed. One week later, a fresh patch having the same content was applied to the same skin site. This cycle was repeated three times, resulting in a total of 9 days' exposure to the patch.

INDUCTION OF ALLERGIC AIRWAY INFLAMMATION

One week after removal of the last patch, each mouse was intranasally challenged with OVA (200 μg in 20 μl of PBS) or PBS alone for 3 days (one challenge per day). Twenty-four hours after the last challenge, bronchoalveolar lavage fluid (BALF) was collected from each mouse, as described elsewhere.[28] The total cell count and leukocyte profile were determined with a hemocytometer (Sysmex XT-1800i; Sysmex Corporation, Hyogo, Japan), as described previously.[29]

MEASUREMENT OF MYELOPEROXIDASE (MPO) AND EOSINOPHIL PEROXIDASE (EPO) ACTIVITIES

MPO and EPO activities were measured as described elsewhere.[28] Recombinant human MPO and EPO (Calbiochem) were used as standard reagents.

QUANTITATIVE REAL-TIME PCR

Twenty-four hours after the last challenge, the lungs were harvested. Total RNA in the lung homogenates was isolated and Quantitative real-time PCR was performed as described elsewhere.[29] The mRNA expression levels were normalized to the GAPDH level in each sample. PCR primers were designed as shown in Table 1.

HISTOLOGY

Twenty-four hours after the last challenge, the lungs were harvested and fixed in Carnoy's solution. The fixed tissue was embedded in paraffin and sliced into 5-μm sections, followed by hematoxylin-eosin or periodic acid-Schiff (PAS) staining.

MEASUREMENT OF OVA-SPECIFIC IMMUNOGLOBULINS IN SERA

Sera were collected twenty-four hours after the last challenge. The level of OVA-specific IgE in each serum was determined by ELISA, as described elsewhere.[30] Anti-OVA mouse IgE (TOS-2), as a standard OVA-specific IgE, was kindly provided by Dr. Mamoru Kiniwa (Taiho Pharmaceutical, Saitama, Ja-

Table 1 Primer designs

Gene	Forward (5'-3')	Reverse (5'-3')
GAPDH	CCCACTCTTCCACCTTCGATG	AGGTCCACCACCCTGTTGCT
ST2 short	TCAACCGCCTAGTGAACACACC	CAAAGCCCAAAGTCCCATTCTC
IL-33	CAGGCCTTCTTCGTCCTTCAC	TCTCCTCCACTAGAGCCAGCTG
IL-4	TCCAAGGTGCTTCGCATATTTT	CAGCTTATCGATGAATCCAGGC
IL-5	CCCTCATCCTCTTCGTTGCAT	ATGTGATCCTCCTGCGTCCAT
IL-13	GGCAGCAGCTTGAGCACATT	GGCATAGGCAGCAAACCATG
IL-17A	CCGCAATGAAGACCCTGATAGAT	AGAATTCATGTGGTGGTCCAGC
CCL11	GAATCACCAACAACAGATGCAC	ATCCTGGACCCACTTCTTCTT
CCL22	ATCCTGGACCCACTTCTTCTT	CGGCAGGATTTTGAGGTCCA
CXCL1	CGGCAGGATTTTGAGGTCCA	TGAACGTCTCTGTCCCGAGC
CXCL2	AACTGACCTGGAAAGGAGGAGC	ACTCTCAGACAGCGAGGCACAT

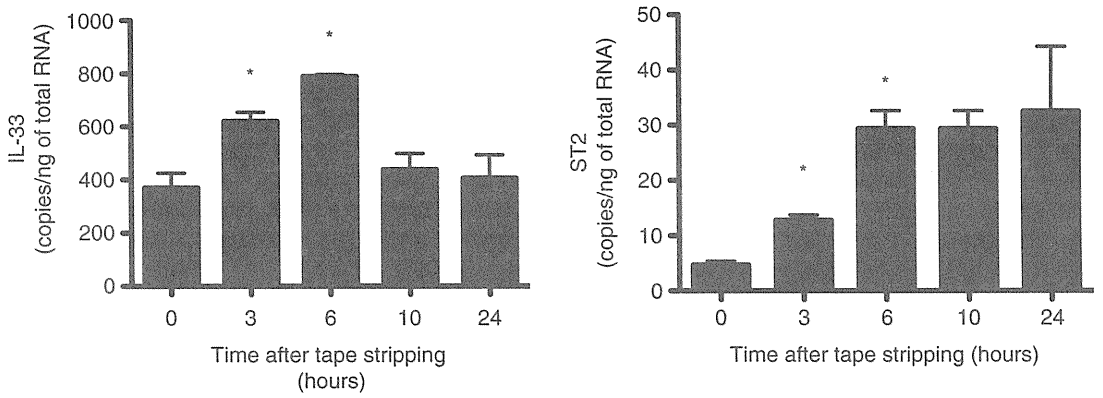

Fig. 1 Expression of IL-33 and ST2 mRNA was upregulated in the skin after tape stripping. mRNA was isolated from the dorsal skin of wild-type mice at the indicated time points after tape stripping. The expression of IL-33 and ST2 mRNA was determined by quantitative PCR. Data show the mean + SE (n = 4). *P < 0.05 vs. 0 (hours).

pan).

STATISTICS

Unless otherwise specified, the unpaired Student's t-test, two-tailed, was used for statistical evaluation of the results. All results are shown as the mean + SEM.

RESULTS

EXPRESSION OF IL-33 AND ST2 mRNA WAS UPREGULATED IN THE SKIN AFTER TAPE STRIPPING

An active form of IL-33 that is localized in the cell nucleus[15] is considered to be released by necrotic cells during tissue injury.[16-18] Patients with atopic dermatitis scratch inflamed skin lesions, resulting in exacerbation of the symptoms due to mechanical skin injury.[31] Since increased expression of IL-33 was observed in the lesions of patients with atopic dermatitis,[19] mechanical skin injury by scratching may result in release of IL-33, contributing to the disease development. Indeed, we found that expression of IL-33 and ST2 mRNA was significantly increased in the skin of wild-type mice at 3 and 6 hours after tape stripping, which was used to mimic mechanical skin injury due to scratching (Fig. 1). These observations suggest that scratching-induced IL-33 and ST2 production may contribute to the pathogenesis of allergic diseases.

ALLERGIC AIRWAY INFLAMMATION WAS ATTENUATED IN ST2$^{-/-}$ MICE SENSITIZED EPICUTANEOUSLY WITH OVA

Allergic airway inflammation was induced by OVA inhalation in mice that had been epicutaneously sensitized with OVA by tape stripping.[10,13] To elucidate the role of the IL-33/ST2 pathway in that setting, we epicutaneously sensitized BALB/c-wild-type (WT) and ST2$^{-/-}$ mice with OVA and then induced airway inflammation by intranasal challenge with OVA, as shown in Figure 2. Pulmonary inflammation accompanied by eosinophil and neutrophil infiltration, goblet cell hyperplasia and mucus secretion, but not epithelial and smooth muscle cell hyperplasia, was observed in epicutaneously OVA-sensitized WT mice af-

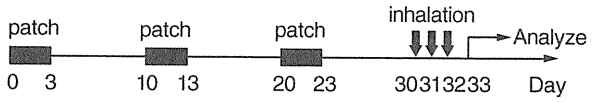

Fig. 2 Experimental protocol.

ter the last OVA, but not PBS, challenge (Fig. 3A, B). On the other hand, epicutaneously OVA-sensitized ST2$^{-/-}$ mice showed suppressed eosinophil and neutrophil infiltration, but not goblet cell hyperplasia and mucus secretion, compared with epicutaneously OVA-sensitized WT mice after the last OVA challenge (Fig. 3A, B). Consistent with this, the numbers of cells such as eosinophils, neutrophils and lymphocytes in BALFs were significantly increased in both epicutaneously OVA-sensitized WT and ST2$^{-/-}$ mice after intranasal challenge with OVA in comparison with after PBS inhalation (Fig. 3C). However, the total-cell, eosinophil and macrophage, but not lymphocyte, counts in BALFs from ST2$^{-/-}$ mice were significantly reduced compared with WT mice (Fig. 3C). The levels of EPO activity in BALFs from ST2$^{-/-}$ mice were also decreased in that setting (Fig. 4A). Despite a slight, but not statistically significant, decrease in neutrophils in BALFs from ST2$^{-/-}$ mice (Fig. 3C), the level of MPO activity was markedly decreased in those BALFs compared with BALFs from WT mice (Fig. 4A). In addition, after the challenge with OVA, the level of OVA-specific IgE in sera from epicutaneously OVA-sensitized ST2$^{-/-}$ mice was also significantly reduced compared with epicutaneously OVA-sensitized WT mice (Fig. 4B).

IL-33-ST2 PATHWAY IS REQUIRED FOR Th2-TYPE, BUT NOT Th17-TYPE, IMMUNE RESPONSES IN MICE AFTER EPICUTANEOUS Ag SENSITIZATION

Others demonstrated that airway eosinophilia is mediated by Th2 cytokines, while airway neutrophilia is mediated by Th17 cytokines, in mice epicutaneously sensitized with OVA and then subjected to intranasal OVA challenge.[11,12] Consistent with previous reports, we observed that-in addition to expression of mRNA for Th2 cytokines (i.e., IL-4, IL-5 and IL-13) and Th2-associated chemokines (i.e., CCL11 and CCL22) the expression of mRNA for IL-17A and neutrophil chemoattractants (i.e., CXCL1 and CXCL2) in the lungs from epicutaneously OVA-sensitized WT mice after the last OVA challenge was significantly increased in comparison with after PBS inhalation (Fig. 5). Consistent with the number of eosinophils and the level of EPO activity in BALFs, mRNA expression for IL-4, IL-5, IL-13, CCL11 and CCL22 in the lungs from epicutaneously OVA-sensitized ST2$^{-/-}$ mice was significantly decreased compared with epicutaneously OVA-sensitized WT mice after the last OVA challenge (Fig. 5). Likewise, expression of mRNA was decreased for CXCL1 and CXCL2 but normal for IL-17A in the lungs from epicutaneously OVA-sensitized ST2$^{-/-}$ mice compared with epicutaneously OVA-sensitized WT mice after the last OVA challenge (Fig. 5). These observations suggest that the IL-33/ST2 pathway is crucial for development of Th2-type immune responses and contributes to, but not is essential for, the development of Th17-type immune responses during antigen sensitization due to mechanical skin injury.

DISCUSSION

IL-33 is known to induce secretion of Th2-type cytokines by various types of cells such as Th2 cells, mast cells, basophils, eosinophils and innate-type lymphoid cells (i.e., natural helper cells, nuocytes, MPP2 cells and ih2 cells) through its ST2 and IL-1RAcP receptors,[14,32-36] suggesting involvement of IL-33 in the pathogenesis of Th2-type allergic disorders. Indeed, the levels of soluble ST2 proteins and IL-33 mRNA/protein are increased in sera and/or tissue specimens from patients with asthma[37-39] and in the lungs of mice that developed allergic airway inflammation.[40,41] Moreover, it is suspected that single-nucleotide polymorphisms in the regions of the *IL-33* and *ST2* genes may influence susceptibility to allergic disorders.[20-26] However, the role of the IL-33/ST2 pathway in the development of OVA-induced allergic airway inflammation in mice remains controversial, as reviewed elsewhere.[14] Following two sensitizations with OVA emulsified with alum (OVA/Alum) and then challenge by OVA inhalation, respiratory function and/or eosinophilic airway inflammation were observed to be normal in ST2$^{-/-}$ mice[42-44] but attenuated in anti-ST2 mAb-treated wild-type mice.[45,46] On the other hand, airway inflammation was attenuated in ST2$^{-/-}$ mice that had been sensitized only once with OVA/alum and then challenged by OVA inhalation.[43] The reason for the discrepancy remains unclear. However, it might be partially due to an immunomodulatory effect of alum, which was used as an adjuvant, on the antigen sensitization. For example, mast cells, B cells, IgE, IL-1, TNF and CCR8 are not required for development of OVA-induced airway inflammation in mice that had been sensitized with OVA/alum, whereas they are essential for that event in mice that had been sensitized with OVA in the absence of alum, as reviewed elsewhere.[47] In the present study, we used another asthma-like mouse model in which mice were sensitized with OVA in the absence of alum via an epicutaneous route after tape stripping of the skin. It is known that airway inflammation observed in epicutaneously antigen-sensitized mice is associated with Th2-type cytokine-mediated eosinophilia and Th17-type cytokine-mediated neutrophilia.[11] Using that model, we clearly demonstrated that development of Th2-type allergic airway inflammation was significantly attenuated in epicutaneously

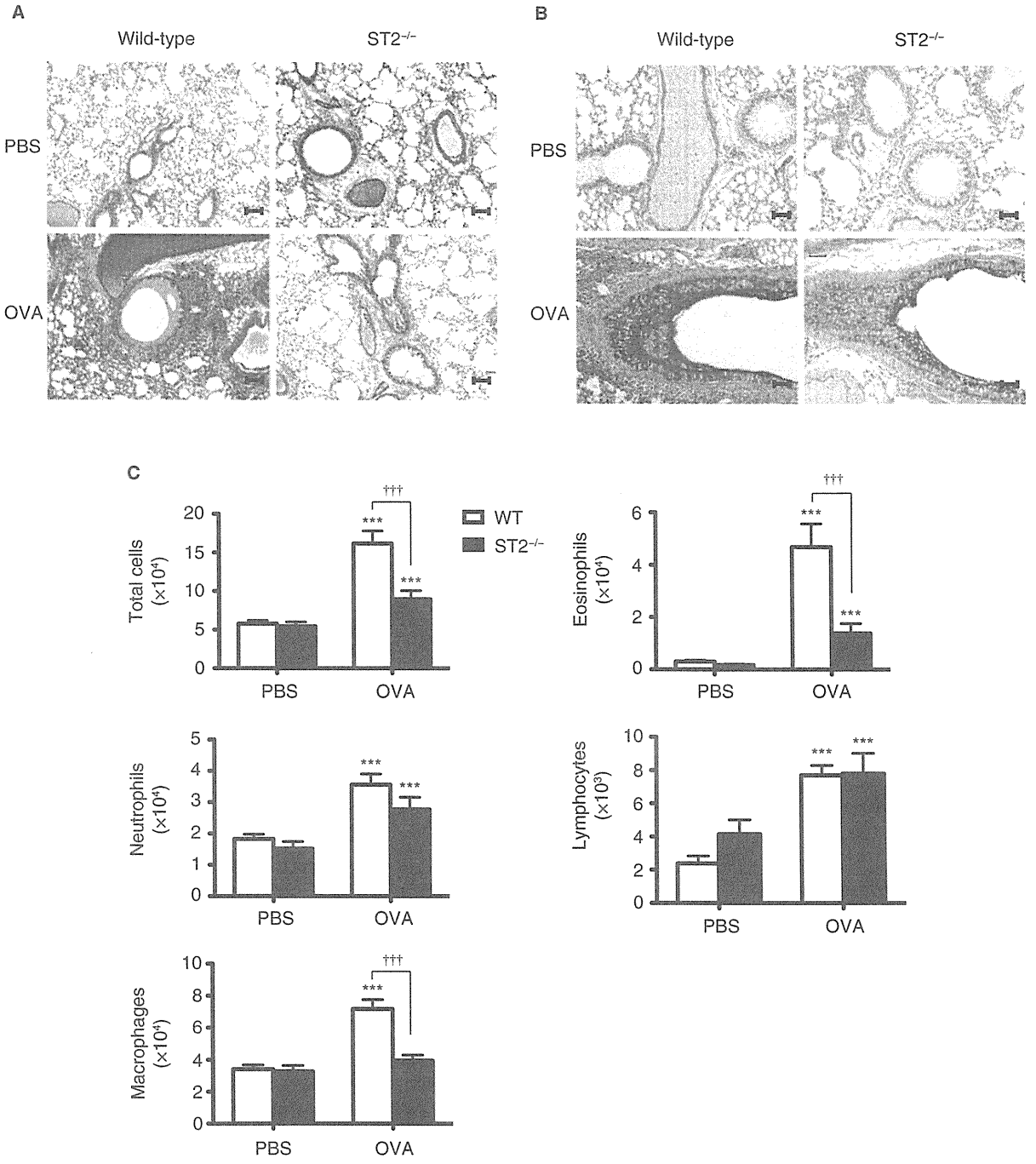

Fig. 3 Pulmonary inflammation was attenuated in epicutaneous OVA-sensitized ST2$^{-/-}$ mice after OVA challenge. Mice were epicutaneously sensitized with OVA, followed by intranasal challenge with OVA or PBS, as shown in Figure 2. Twenty-four hours after the last OVA or PBS inhalation, the lungs, BAL cells and fluids, and sera were collected. (**A, B**) Lung histology. H&E (**A**) and PAS (**B**) staining. Bar = 100 μm. The data show representative results from 8-12 mice in each experimental group, as indicated. (**C**) The number of BAL cells. The data show the mean + SEM (wild-type [WT] mice: PBS, n = 16, and OVA, n = 20; ST2$^{-/-}$ mice: PBS, n = 14, and OVA, n = 20). ***$P < 0.001$ vs. the corresponding values for PBS-treated mice, and $^{\dagger\dagger\dagger}P < 0.001$ vs. the indicated group.

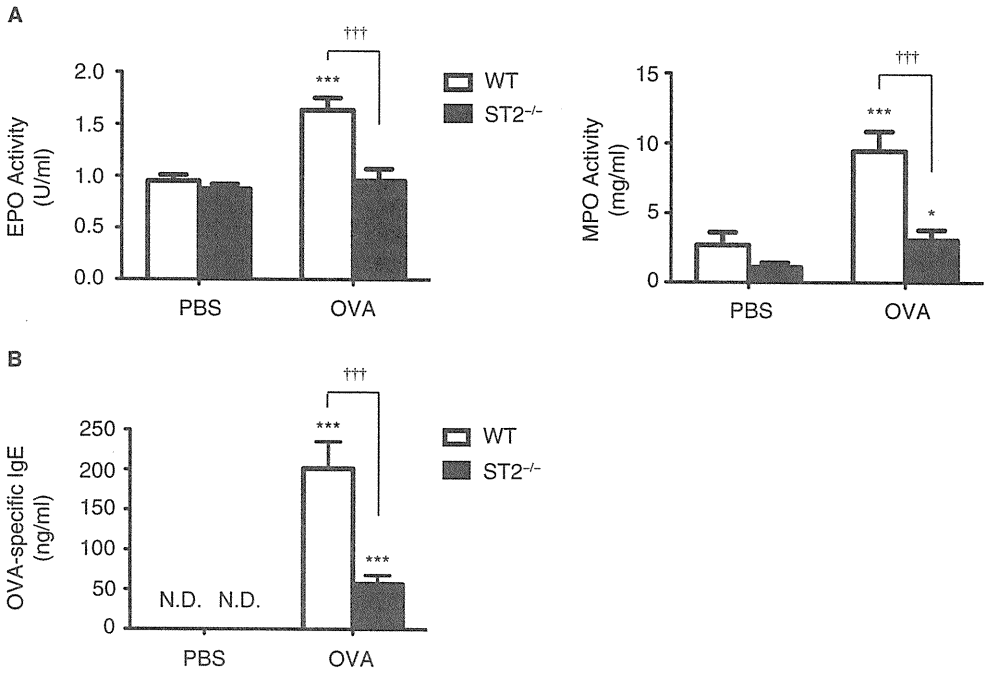

Fig. 4 The levels of EPO and MPO activities in BALFs and serum IgE were reduced in epicutaneous OVA-sensitized ST2$^{-/-}$ mice after OVA challenge. (**A**) The levels of EPO and MPO activities in BALFs and (**B**) the levels of OVA-specific IgE in sera of the wild-type (WT) and ST2$^{-/-}$ mice used in Figure 3B. * $P < 0.05$ and *** $P < 0.001$ vs. the corresponding values for PBS-treated mice, and ††† $P < 0.001$ vs. the indicated groups.

OVA-sensitized ST2$^{-/-}$ mice after intranasal OVA challenge (Fig. 3B, 4A, 5), suggesting that the IL-33/ST2 pathway is crucial for induction of Th2-type allergic airway inflammation, especially under adjuvant-free experimental conditions.

It was recently reported that Th2-type eosinophilic airway inflammation after OVA inhalation was dramatically suppressed in IL-4$^{-/-}$ IL-13$^{-/-}$ mutant mice that had been epicutaneously sensitized with OVA, whereas Th17-type neutrophilic airway inflammation was exaggerated.[12] IL-17 production by T cells can be inhibited by both IL-4 and IFN-γ,[48] suggesting that IL-4-deficiency resulted in increased IL-17 production and led to the enhanced IL-17-mediated immune responses observed in IL-4$^{-/-}$ IL-13$^{-/-}$ mice. In contrast to IL-4$^{-/-}$ IL-13$^{-/-}$ mice,[12] we found normal levels of pulmonary IL-17 mRNA expression and airway neutrophilia in epicutaneously OVA-sensitized ST2$^{-/-}$ mice after the last OVA challenge (Fig. 3B, 5). It may be explained that IL-4 mRNA expression was significantly decreased, but not completely abrogated, in ST2$^{-/-}$ mice (Fig. 5).

Despite the normal airway neutrophilia and IL-17 levels described above, the levels of CXCL1 and CXCL2 mRNA expression and MPO activity were markedly decreased in lungs and BALFs, respectively, from epicutaneously OVA-sensitized ST2$^{-/-}$ mice after the last OVA challenge (Fig. 4A, 5). This suggests that the IL-33/ST2 pathway may be partially involved in neutrophil recruitment, independently of IL-17. Indeed, we previously reported that IL-33 can directly induce production of neutrophil chemoattractants (i.e., IL-8) by both endothelial and epithelial cells.[49] However, since the effect of the IL-33/ST2 pathway on neutrophil recruitment was less than that of IL-17, ST2 deficiency may not exert a significant effect on airway neutrophilia in our experimental system.

In summary, we demonstrated that the IL-33/ST2 pathway is crucial for Th2-cytokine-mediated eosinophilic, rather than Th17-cytokine-mediated neutrophilic, airway inflammation in mice that had been epicutaneously sensitized with antigen. Our findings may provide a clue for development of novel therapeutics for asthma.

ACKNOWLEDGEMENTS

We thank Dr. Andrew N.J. McKenzie (MRC Laboratory of Molecular Biology, Cambridge, United Kingdom) for providing ST2$^{-/-}$ mice, and Shuhei Fukuda, Noriko Hashimoto, Yoshiko Shimamoto and Hiromi Wakita for their excellent technical assistance, and Larry Stiver for critical reading of the manuscript. This work was supported by a grant from the Na-

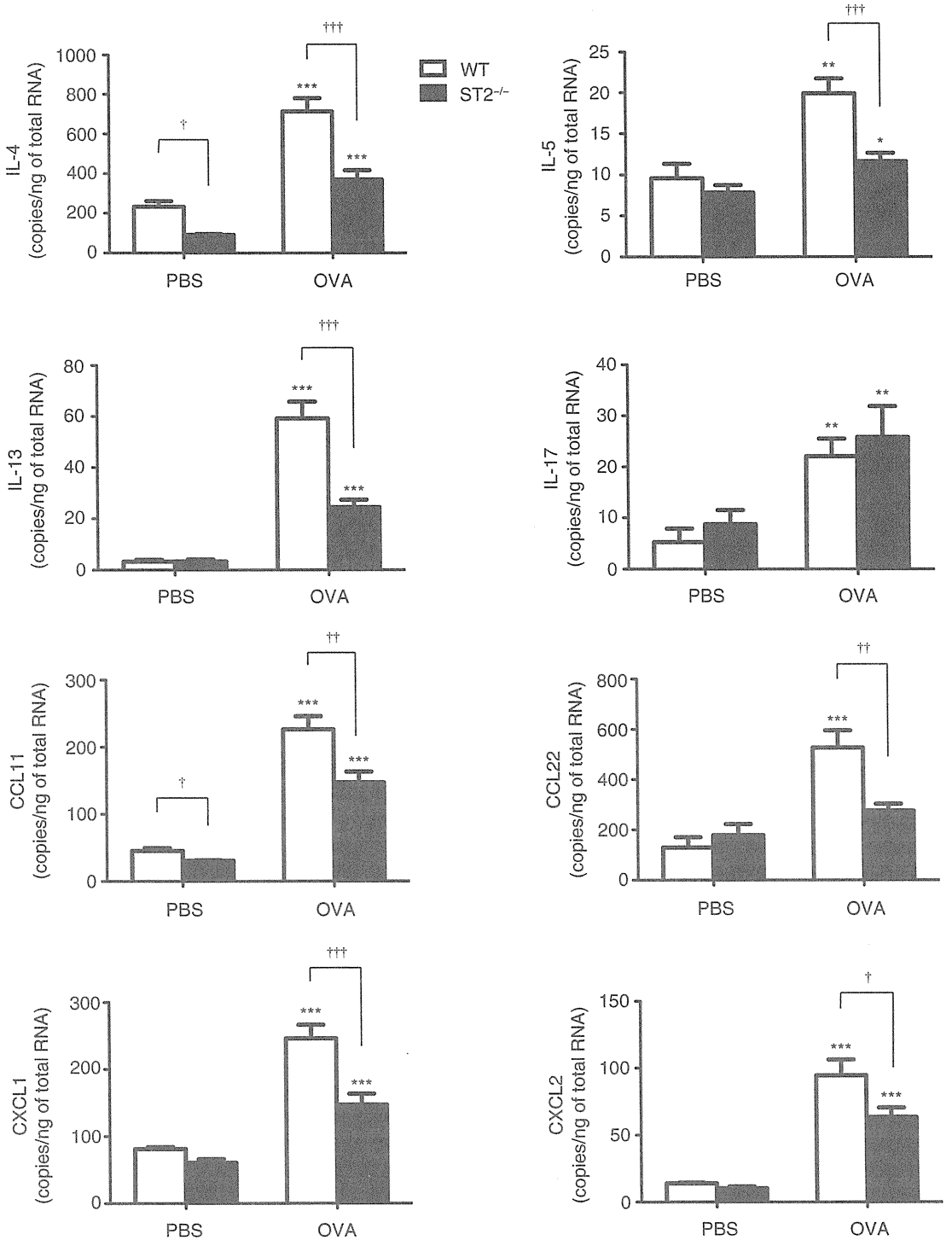

Fig. 5 The expression levels of Th2 cytokines, but not IL-17A, were reduced in lungs from epicutaneous OVA-sensitized ST2$^{-/-}$ mice after OVA challenge. Twenty-four hours after the last OVA challenge, lungs were harvested from the wild-type (WT) and ST2$^{-/-}$ mice used in Figure 3B, and the total mRNA was isolated. The expression of mRNA for cytokines and chemokines was determined by quantitative PCR. Data show the mean + SEM (WT mice: PBS, $n = 16$, and OVA, $n = 20$; ST2$^{-/-}$ mice: PBS, $n = 14$, and OVA, $n = 20$). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. the corresponding values for PBS-treated mice, and $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$ vs. the indicated groups.

tional Institute of Biomedical Innovation, a Research on Allergic Disease and Immunology in Health Labour Sciences Research Grant, a Grant for Child Health and Development from the Ministry of Health, Labour and Welfare, and a Grant-in-Aid for Scientific Research (B) and the Program for Improvement of Research Environment for Young Researchers, The Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

1. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;**112**:S118-27.
2. Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: A birth cohort study of subjects at risk. *J Allergy Clin Immunol* 2001; **108**:720-5.
3. Rhodes HL, Thomas P, Sporik R, Holgate ST, Cogswell JJ. A birth cohort study of subjects at risk of atopy: Twenty-two-year follow-up of wheeze and atopic status. *Am J Respir Crit Care Med* 2002;**165**:176-80.
4. Gustafsson D, Sjoberg O, Foucard T. Development of allergies and asthma in infants and young children with atopic dermatitis—a prospective follow-up to 7 years of age. *Allergy* 2000;**55**:240-5.
5. Weidinger S, Illig T, Baurecht H *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006;**118**:214-9.
6. Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;**38**:441-6.
7. Weidinger S, O'Sullivan M, Illig T *et al.* Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008;**121**:1203-9.
8. Marenholz I, Kerscher T, Bauerfeind A *et al.* An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. *J Allergy Clin Immunol* 2009;**123**:911-6.
9. Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit Th17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009;**124**:485-93.
10. Kodama M, Asano K, Oguma T *et al.* Strain-specific phenotypes of airway inflammation and bronchial hyperresponsiveness induced by epicutaneous allergen sensitization in BALB/c and C57BL/6 mice. *Int Arch Allergy Immunol* 2010;**152**:67-74.
11. He R, Oyoshi MK, Jin H, Geha RS. Epicutaneous antigen exposure induces a Th17 response that drives airway inflammation after inhalation challenge. *Proc Natl Acad Sci U S A* 2007;**104**:15817-22.
12. He R, Kim HY, Yoon J *et al.* Exaggerated IL-17 response to epicutaneous sensitization mediates airway inflammation in the absence of IL-4 and IL-13. *J Allergy Clin Immunol* 2009;**124**:761-70.
13. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;**101**:1614-22.
14. Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S. IL-33 and IL-33 receptors in host defense and diseases. *Allergol Int* 2010;**59**:143-60.
15. Baekkevold ES, Roussigne M, Yamanaka T *et al.* Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol* 2003;**163**:69-79.
16. Luthi AU, Cullen SP, McNeela EA *et al.* Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 2009;**31**:84-98.
17. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A* 2009;**106**:9021-6.
18. Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* 2009;**284**:19420-6.
19. Pushparaj PN, Tay HK, H'Ng S C *et al.* The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 2009;**106**:9773-8.
20. Gudbjartsson DF, Bjornsdottir US, Halapi E *et al.* Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; **41**:342-7.
21. Moffatt MF, Gut IG, Demenais F *et al.* A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;**363**:1211-21.
22. Reijmerink NE, Postma DS, Bruinenberg M *et al.* Association of IL1RL1, IL-18R1, and IL-18RAP gene cluster polymorphisms with asthma and atopy. *J Allergy Clin Immunol* 2008;**122**:651-4.
23. Shimizu M, Matsuda A, Yanagisawa K *et al.* Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. *Hum Mol Genet* 2005;**14**:2919-27.
24. Ali M, Zhang G, Thomas WR *et al.* Investigations into the role of ST2 in acute asthma in children. *Tissue Antigens* 2009;**73**:206-12.
25. Castano R, Bosse Y, Endam LM, Desrosiers M. Evidence of association of interleukin-1 receptor-like 1 gene polymorphisms with chronic rhinosinusitis. *Am J Rhinol Allergy* 2009;**23**:377-84.
26. Sakashita M, Yoshimoto T, Hirota T *et al.* Association of serum interleukin-33 level and the interleukin-33 genetic variant with japanese cedar pollinosis. *Clin Exp Allergy* 2008;**38**:1875-81.
27. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;**191**:1069-76.
28. Nakae S, Lunderius C, Ho LH, Schafer B, Tsai M, Galli SJ. TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. *J Allergy Clin Immunol* 2007;**119**:680-6.
29. Oboki K, Ohno T, Kajiwara N *et al.* IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010;**107**:18581-6.
30. Nakae S, Suto H, Berry GJ, Galli SJ. Mast cell-derived tnf can promote Th17 cell-dependent neutrophil recruitment in ovalbumin-challenged otii mice. *Blood* 2007;**109**:3640-8.
31. Wahlgren CF. Itch and atopic dermatitis: An overview. *J Dermatol* 1999;**26**:770-9.
32. Schmitz J, Owyang A, Oldham E *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;**23**:479-90.

33. Guo L, Wei G, Zhu J *et al.* IL-1 family members and stat activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci U S A* 2009;**106**:13463-8.
34. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* 2009;**113**:1526-34.
35. Ho LH, Ohno T, Oboki K *et al.* IL-33 induces IL-13 production by mouse mast cells independently of igefcepsilonri signals. *J Leukoc Biol* 2007;**82**:1481-90.
36. Moro K, Yamada T, Tanabe M *et al.* Innate production of T(h)2 cytokines by adipose tissue-associated c-kit(+) sca-1(+) lymphoid cells. *Nature* 2010;**463**:540-4.
37. Kuroiwa K, Li H, Tago K *et al.* Construction of elisa system to quantify human ST2 protein in sera of patients. *Hybridoma* 2000;**19**:151-9.
38. Oshikawa K, Kuroiwa K, Tago K *et al.* Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med* 2001;**164**: 277-81.
39. Prefontaine D, Lajoie-Kadoch S, Foley S *et al.* Increased expression of IL-33 in severe asthma: Evidence of expression by airway smooth muscle cells. *J Immunol* 2009;**183**: 5094-103.
40. Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. Expression and function of the ST2 gene in a murine model of allergic airway inflammation. *Clin Exp Allergy* 2002;**32**: 1520-6.
41. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007;**282**:26369-80.
42. Hoshino K, Kashiwamura S, Kuribayashi K *et al.* The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J Exp Med* 1999;**190**:1541-8.
43. Kurowska-Stolarska M, Kewin P, Murphy G *et al.* Il-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 2008;**181**:4780-90.
44. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. *Eur J Immunol* 2007;**37**:1302-12.
45. Meisel C, Bonhagen K, Lohning M *et al.* Regulation and function of T1/ST2 expression on CD4+ t cells: Induction of type 2 cytokine production by T1/ST2 cross-linking. *J Immunol* 2001;**166**:3143-50.
46. Coyle AJ, Lloyd C, Tian J *et al.* Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J Exp Med* 1999;**190**:895-902.
47. Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int* 2008;**57**:121-34.
48. Dong C. Diversification of T-helper-cell lineages: Finding the family root of IL-17-producing cells. *Nat Rev Immunol* 2006;**6**:329-33.
49. Yagami A, Orihara K, Morita H *et al.* IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010;**185**:5743-50.

Allergology International. 2011;60:509-515
DOI: 10.2332/allergolint.10-OA-0293

ORIGINAL ARTICLE

Age-related Prevalence of Allergic Diseases in Tokyo Schoolchildren

Masaki Futamura[1,2], Yukihiro Ohya[1,2], Masayuki Akashi[1,2], Yuichi Adachi[2,3], Hiroshi Odajima[2,4], Kazuo Akiyama[2,5] and Akira Akasawa[2,6]

ABSTRACT

Background: The International Study of Asthma and Allergies in Childhood (ISAAC) has reported the prevalence of asthma and allergic diseases in many countries.

Methods: We used the ISAAC core written questionnaire to examine the prevalence of asthma and allergic diseases in 6- to 14-year old schoolchildren in Tokyo. In 2005, we conducted a cross-sectional survey of all schoolchildren in all public schools located in the Setagaya area of Tokyo.

Results: Data were collected from 27,196 children in 95 schools. Prevalence ranged from 10.5% to 18.2% for asthma symptoms and from 10.9% to 19.6% for atopic dermatitis, with both conditions tending to decrease with age. As has been previously reported for all age groups, significantly higher rates of current asthma are observed in boys than in girls. The prevalence of allergic rhinoconjunctivitis exhibited a different pattern from that of asthma and atopic dermatitis, peaking at the age of 10 (34.8%). Prevalence of allergic rhinoconjunctivitis was 1.5 to 2-fold higher than the previous ISAAC studies that were performed in Tochigi and Fukuoka. In all age groups, symptoms of allergic conjunctivitis were more frequent from February to May, which coincides with the Japanese cedar pollen season, and were less frequent between June to September.

Conclusions: The prevalence of asthma and atopic dermatitis was higher in younger schoolchildren. Tokyo schoolchildren appear to have extremely high prevalence rates of seasonal allergic rhinoconjunctivitis.

KEY WORDS

asthma, atopic dermatitis, ISAAC, prevalence, rhinitis

INTRODUCTION

Asthma and allergic diseases are common in children. Urbanization has led to an increase in allergic diseases, and thus, this has become an important health problem in today's society.

Many countries have conducted large prevalence surveys of asthma and allergic diseases. In 1991, the International Study of Asthma and Allergic diseases in Childhood (ISAAC) established a standardized methodology to compare the prevalence and severity of asthma and atopic diseases in children.[1] Since starting the study in 1993, the ISAAC Phase One study group has examined the prevalence rates of asthma in children around the world and found that over a 12-month period, the highest rates were for children living in the UK, Australia, and New Zealand, while the lowest were in children residing in Eastern Europe, the Asia-Pacific, and Africa.[2] In contrast, the highest prevalence rates of symptoms related to allergic rhinoconjunctivitis occurred in countries that were scattered across the world. Moreover, the prevalence of allergic rhinoconjunctivitis in children aged 13-14 years was higher than those aged 6-7 years in all of the countries studied around the world. Interestingly, this pattern was not seen for asthma.

The Japanese Ministry of Education, Culture, Sports, Science and Technology has announced that the prevalence of doctor-diagnosed asthma in children has doubled from 1994 to 2004. They also reported that schoolchildren are more likely than other age groups to develop asthma.[3]

[1]Division of Allergy, Department of Medical Specialties, National Center for Child Health and Development, [2]Japanese Asthma Survey Group, [6]Division of Allergy, Tokyo Metropolitan Children's Medical Center, Tokyo, [3]Department of Pediatrics, University of Toyama, Toyama, [4]Division of Pediatrics, Fukuoka National Hospital, Fukuoka and [5]Sagamihara National Hospital, Kanagawa, Japan.
Correspondence: Masaki Futamura, MD, PhD, Division of Allergy, Department of Medical Subspecialties, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan.
Email: futamura-m@ncchd.go.jp
Received 16 November 2010. Accepted for publication 12 March 2011.

In Japan, ISAAC surveys have been conducted in Fukuoka, the eighth largest city in Japan, and in Tochigi, which is an average-sized city. In Tochigi in 1995, the prevalence of allergic rhinoconjunctivitis in children aged 13-14 was 21.5%.[4] In Fukuoka, the 12-month prevalence of allergic rhinoconjunctivitis among children aged 6-7 increased from 7.8% in 1994 to 10.6% in 2002, while over the same period of time in children aged 13-14, it increased from 14.9% to 17.6%.[5] Similar trends were also seen in many other Asia-Pacific countries and in India. In contrast, the prevalence of asthma, however, did not notably increase in any of these countries or in Japan.

Children born in urban areas are expected to have higher prevalences of allergic diseases than those born in rural areas.[6] Although Tokyo is the largest city in Japan, and thereby, would be expected to have the highest prevalence of allergic diseases, previous ISAAC surveys have never been done in this city. Thus, there were two aims of the present study. First, the ISAAC protocol was used to determine whether age-related differences are responsible for the prevalence of allergic symptoms observed among Tokyo schoolchildren. Based on these findings, the second part of the study was designed to compare these results with the findings of previous ISAAC studies in Japan and determine if there were differences between large urban areas and other areas that were less populated/more rural in nature.

METHODS

SUBJECTS

The survey was conducted from May to June, 2005, in accordance with the ISAAC protocol.[7] The present survey was part of an investigation by the Japanese Asthma Survey Group (JASG), and was aimed at surveying the prevalence of allergic diseases in all age groups at various places throughout Japan.

Setagaya was chosen as the research zone for this study, as it is located in the center of Tokyo. During the study period, this was the biggest geographical region within the Greater Tokyo Area. Setagaya has a population density that is close to the Tokyo average, with 830,000 inhabitants living in about 58 km^2 (22 square miles).

In Japan, compulsory education consists of nine grades (years). In April of each year, children who have reached the age of 6 enroll in an elementary school that has six grades. After graduating from elementary school, students enter junior high school, which has three grades. The current survey covered all of the schoolchildren in these nine grades. During the study period, Setagaya had 64 public elementary schools and 31 public junior high schools, with approximately 80% of the children attending these public schools. With the help of the Setagaya City Board of Education, we were able to investigate all public elementary and junior high school students.

QUESTIONNAIRE

We used the ISAAC written questionnaire for 6-7 year olds for the elementary school children and the questionnaire for 13-14 year olds for the junior high school children. Our group previously translated the ISAAC written questionnaire from English into Japanese and then back into English to confirm its accuracy. An explanatory note for eczema and rash was added, as the Japanese language does not normally differentiate between the two. The questionnaire was distributed at all of the schools, with the children then taking it home to be filled out. Prior to filling out the questionnaire, all participants in the study provided informed consent. For the younger age group, the children's parents completed the questionnaire, while the children in the older age group completed it on their own. After completing the form, the questionnaires were taken back to the schools for collection.

Based on the questionnaire answers, we evaluated the 12-month point prevalences of asthma, allergic rhinoconjunctivitis, and atopic dermatitis.[7] To define current asthma and examine wheezing during the previous 12 months, we asked the following question, "Have you (has your child) had wheezing or whistling in the chest in the last 12 months?". If current asthma was present, the questionnaire further assessed the frequency and severity of the episodes. Questions pertaining to allergic rhinoconjunctivitis included those regarding sneezing or a running or blocked nose (in the absence of flu) that was associated with itchy-watery eyes over the last 12 months. The monthly frequency among children who had symptoms of allergic rhinoconjunctivitis was evaluated by asking, "In which of the past 12 months did this nose problem occur? (please tick any which apply)." Atopic dermatitis was considered to be present when there was an itchy, relapsing skin rash that affected the flexural areas during the preceding 12 months.

ETHICAL CONSIDERATIONS

The ethics committee of the National Center for Child Health and Development approved the study protocol. The older children directly provided informed consent. However, since parents completed the questionnaire for the younger children, parental informed consent was obtained in this group.

STATISTICAL ANALYSIS

Analyses focused on changes in the 12-month prevalence of the symptoms, which included asthma, rhinitis and dermatitis. Data were analyzed using SPSS 15.0J (SPSS Inc., Chicago, IL, USA), with a p-value of <0.05 defined as being statistically significant. Proportions between the two groups were compared using chi-squared tests. The interrelationship between age and the 12-month point prevalence was evaluated by Pearson's correlation.

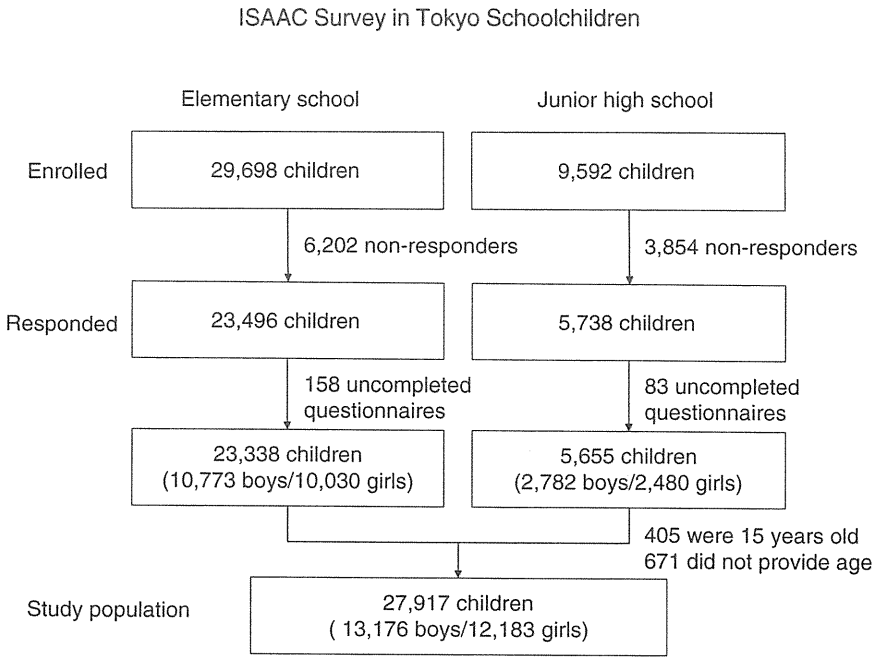

Fig. 1 Study subjects and the study protocol. All students in all public schools in Setagaya from May through June of 2005 were enrolled in the study, with more than 70% of all children aged 6-14 years at these schools included in the analyses.

RESULTS

Of the 95 schools approached, all agreed to participate, which resulted in a target population of 39,290 children (Fig. 1). Out of this population, a total of 23,338 elementary school children (78.6%), aged 6 to 12 years, and 5,655 junior high school children (59.0%), aged 12 to 15 years, completed questionnaires. For the 15-year-old children, numbers were quite small and thus, we excluded this group from the analyses. Of the 27,917 children aged 6 to 14 that we were able to analyze, 13,176 (47.2%) were boys, and 12,183 (43.7%) were girls. In 2,558 children, we were not able to determine the gender.

Current asthma prevalence ranged from 10.5% to 18.2% among all age groups (Table 1), with the highest found in the younger children. There was a strong inverse correlation between the age and prevalence (r = -0.956, P < .001). When boys were compared to girls in all of the age groups, boys had significantly higher rates of current asthma (P < .001 for ages 6 to 12, P < .05 for ages 13 and 14). While frequent wheezing and sleep disturbance were more common in younger children and in boys, exercise-induced wheezing during the last 12 months was more common in older children of both sexes and in younger boys.

In contrast to the asthma findings, the prevalence of allergic rhinoconjunctivitis tended to be higher in older children (Table 2), increasing rapidly from age 6 to 10. By the age of 10, the prevalence of allergic rhinoconjunctivitis was 34.8%. In all age groups, symptoms of allergic rhinoconjunctivitis were more frequent from February to May, and less frequent from June to September (Fig. 2). On a day-to-day basis, moderate or severe interference due to rhinoconjunctivitis was more common in older children of both sexes and in boys.

Similar to the asthma findings, the prevalence of atopic dermatitis was highest in younger children, with analyses showing a significant inverse correlation with age (r = -0.983, P < .001) (Table 2). However, severe symptoms of atopic dermatitis were more often observed in older children. There were no gender differences noted for the prevalence or severity of atopic dermatitis.

Among the 27,389 children aged 6 to 14 years who completed questionnaires about their current asthma, allergic rhinoconjunctivitis and atopic dermatitis symptoms, 14.0% had current asthma. Of these children, 41.6% and 31.3% had the symptoms of allergic rhinoconjunctivitis and atopic dermatitis, respectively. While 43.1% of the children in this study had ≥1 of the symptoms during the past 12 months, only 2.2% had all three symptoms (Fig. 3). Table 3 shows the overlap of the current symptoms for three diseases based on age among the 27,389 children.

DISCUSSION

In 2005 we examined the prevalence of asthma, allergic rhinoconjunctivitis and atopic dermatitis in a large sample of schoolchildren who resided in the Tokyo metropolitan area of Setagaya. The prevalence of current asthma and atopic dermatitis was inversely correlated with age, whereas that of allergic rhinoconjunctivitis showed an age-dependent increase until

Table 1 Prevalence (%) and severity of asthma symptoms in 6 to 14 year old children

Symptoms	Age (years)								
	6	7	8	9	10	11	12	13	14
Current wheeze									
Total	18.2	15.7	15.6	13.3	14.5	11.9	12.0	10.3	10.5
Boys	***21.4	***17.8	***17.8	***16.0	***17.4	***14.7	***14.9	*12.0	*12.0
Girls	14.5	13.5	12.9	10.9	10.9	9.3	9.3	8.6	8.8
Wheezing attacks ≥ 4 /12 months									
Total	5.1	3.7	4.4	3.7	4.0	3.3	3.1	3.3	3.1
Boys	*6.0	**4.6	**5.5	*4.4	*4.6	*4.2	*4.0	4.1	3.1
Girls	4.3	2.8	3.2	2.9	3.0	2.7	2.3	2.7	3.0
Awakened by wheezing ≥ 1 /wk									
Total	2.4	2.3	2.0	2.0	1.6	1.3	1.2	0.6	1.2
Boys	3.0	2.4	2.2	2.1	1.8	*1.6	1.7	0.6	*2.0
Girls	2.0	2.2	1.7	1.9	1.2	0.8	0.9	0.7	0.5
Speech limitation									
Total	2.4	1.7	1.5	1.8	1.2	1.2	1.4	0.9	1.8
Boys	**3.3	2.0	1.8	2.2	*1.7	***1.8	***2.0	1.2	1.9
Girls	1.6	1.6	1.1	1.5	0.8	0.5	1.0	0.7	2.0
Exercised-induced wheezing									
Total	5.8	6.0	6.2	6.7	7.6	7.1	11.7	13.2	14.4
Boys	***7.6	***7.3	6.6	**7.9	7.8	7.9	12.0	13.6	*14.3
Girls	4.1	4.6	5.7	5.3	6.8	6.4	12.1	13.1	15.0

Comparisons were performed between boys and girls for each symptom and age. *P < .05, **P < .01, ***P < .001.

Table 2 Prevalence (%) and severity of allergic rhinoconjunctivitis and atopic dermatitis symptoms in 6 to 14 year old children

Symptoms	Age (years)								
	6	7	8	9	10	11	12	13	14
Allergic rhinoconjunctivitis									
Total	19.7	22.5	25.1	26.9	34.8	32.5	33.8	27.8	29.1
Boys	*21.2	23.1	26.3	27.1	35.6	33.0	35.2	29.3	27.0
Girls	17.7	22.3	23.9	25.4	34.5	31.9	32.3	27.1	30.1
Moderate to severe interference by rhinitis									
Total	10.2	11.9	14.1	14.6	21.4	19.9	21.5	18.1	20.6
Boys	11.2	*13.1	**16.1	15.1	*23.4	*21.1	*23.8	18.9	20.6
Girls	9.5	10.7	12.3	13.3	20.6	18.1	19.4	15.9	19.8
Atopic dermatitis									
Total	19.6	17.4	16.9	16.6	15.3	15.0	13.6	11.9	10.9
Boys	19.9	17.0	17.3	17.7	15.6	14.8	13.3	10.7	10.3
Girls	19.7	18.5	16.2	15.6	14.8	15.0	14.1	12.8	11.4
Kept awake by rash ≥1/wk									
Total	1.6	1.7	1.7	1.5	1.5	1.3	1.9	2.5	2.5
Boys	1.5	1.8	1.9	1.6	1.2	1.2	2.1	2.0	2.7
Girls	1.8	1.6	1.4	1.5	1.4	1.5	1.6	2.9	2.2

Comparisons were performed between boys and girls for each symptom and age. *P < .05, **P < .01, ***P < .001.

reaching the age of 10. These correlations also showed an overlap of the prevalences for the three diseases in accordance with age. These findings suggest that the peak prevalence of asthma and atopic dermatitis may occur at or before the age of 5, similar to that previously reported. In an Australian study, the frequency of atopic dermatitis increased and reached a maximum prevalence by the age of 1, after which it decreased in an age-dependent manner in a group of preschool-age children.[8] For asthma, the

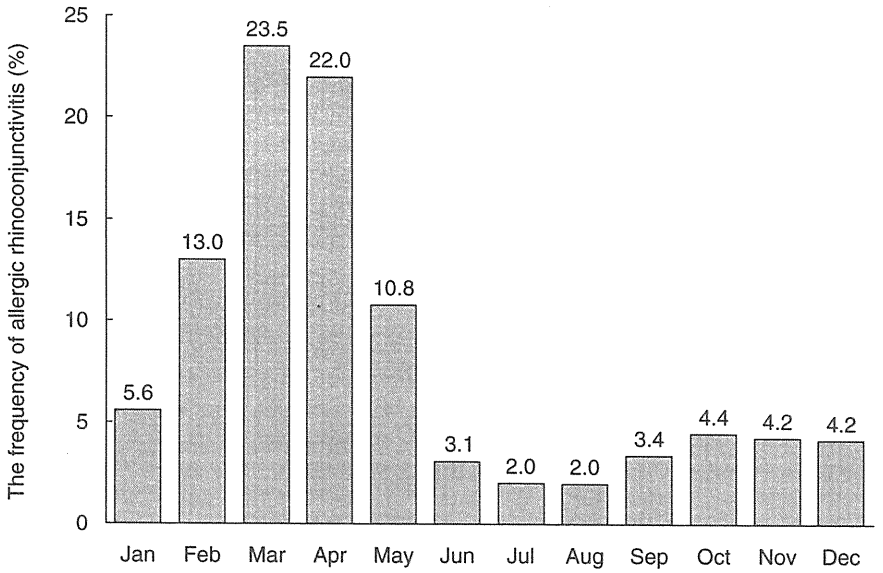

Fig. 2 Monthly frequency of allergic rhinoconjunctivitis in all subject age groups.

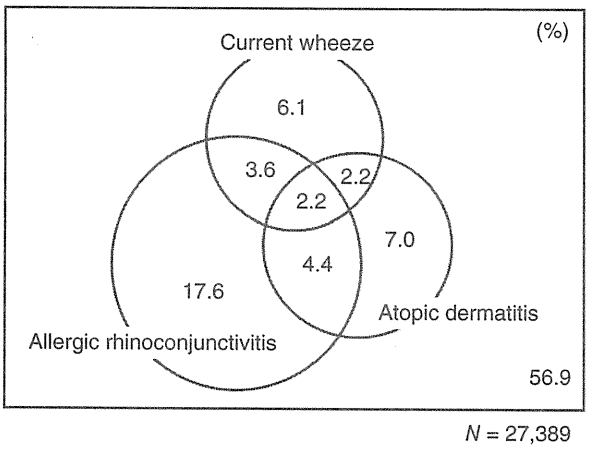

Fig. 3 Venn diagram showing overlap of the current asthma, allergic rhinoconjunctivitis and/or atopic dermatitis symptoms among children aged 6 to 14 who completed questionnaires that gathered symptom prevalence data over a 12-month period.

prevalence in boys was higher than that in girls in each age group of the present study. This supports the findings of previous ISAAC studies, including one that was performed in Fukuoka. This study demonstrated that 6-7 year olds had higher prevalences of asthma and atopic dermatitis and a lower prevalence of allergic rhinoconjunctivitis when compared to 13-14 year olds. The study also showed that the prevalence of asthma was higher in boys.[2]

In the present study, the prevalence of atopic dermatitis also decreased with increasing age. On the other hand, older children were more likely to have severe symptoms. This suggests that even though mild dermatitis appears to have been completely resolved, severe dermatitis actually became exacerbated with increased age. While results of previous ISAAC studies have been mixed with regard to these findings, most Asian studies have shown a similar pattern. Therefore, atopic dermatitis rates in Setagaya, as in other Asian cities, might be influenced by exposure to irritant gases such as car exhaust fumes or by high concentrations of house dust mites.[9]

The prevalence rates of allergic rhinoconjunctivitis in children aged 6-7 and 13-14 in the present study were extremely high as compared to those in the 2002 Fukuoka study, even though the asthma rates were similar. It should also be noted that the prevalence of allergic rhinoconjunctivitis in Tokyo children aged 6-7 was one of the highest that has been documented among all of the ISAAC Phase Three populations.[6] Tokyo has the highest per-capita income in Japan, and thus, our findings are consistent with previous reports that have shown that the prevalence of allergic rhinoconjunctivitis is higher in high-income versus low-income countries.[10]

We compared the present results from Tokyo with those obtained using the same questionnaire several years previously in Fukuoka and Tochigi. These ISAAC Phase Three surveys revealed that the prevalence of allergic diseases in Fukuoka and other Asian areas did not change markedly from Phase One.[11] This suggests that the prevalence of allergic disease in Tokyo is also likely to have remained relatively constant during this period, hence comparisons between the present and previous studies can provide meaningful information.

Table 3 Overlap prevalence (%) of asthma, allergic rhinoconjunctivitis and/or atopic dermatitis symptoms in children 6 to 14 years of age

Symptoms	Age (years)								
	6	7	8	9	10	11	12	13	14
BA (+), ARC (+), AD (+)	2.6	2.1	2.9	2.6	2.2	1.9	1.7	1.6	1.6
BA (+), ARC (+), AD (-)	3.2	3.9	3.4	3.4	4.9	3.4	4.2	2.4	2.5
BA (+), ARC (-), AD (+)	3.5	2.9	2.5	2.1	1.7	1.6	1.3	1.5	1.2
BA (-), ARC (+), AD (+)	3.8	4.0	4.1	4.5	5.5	5.5	5.1	2.7	2.6
BA (+), ARC (-), AD (-)	8.9	6.7	6.8	5.3	5.8	5.0	5.0	4.6	5.1
BA (-), ARC (+), AD (-)	10.0	12.5	14.7	16.4	22.3	21.7	22.9	20.9	22.5
BA (-), ARC (-), AD (+)	9.6	8.4	7.4	7.3	5.9	6.0	5.5	6.1	5.4
BA (-), ARC (-), AD (-)	58.4	59.4	58.1	58.4	51.8	54.8	54.4	60.2	59.1

BA, current wheeze; ARC, allergic rhinoconjunctivitis; AD, atopic dermatitis.

Asthma is more prevalent in urbanized areas, as air pollution is one of environmental factors that can exacerbate asthma. It is well known that components of diesel exhaust particles worsen respiratory symptoms through a variety of mechanisms.[12] In Tokyo, the number of diesel-powered automobiles is heavily regulated by prefectural ordinances that were put in place in 2003 in order to control the severe air pollution. Pollution concentration differences might explain why the prevalence of current wheezing in the present study was higher in Tokyo than in Tochigi, which is a less populated area.

Compared to Fukuoka, Tokyo has higher pollen levels. Thus, the higher exposure to pollen in Tokyo might contribute to the higher prevalence of allergic diseases that are seen as compared to Fukuoka. However, it is unclear as to why Tochigi, which has higher recorded pollen counts would have a lower prevalence of allergic diseases as compared to Tokyo.[13] Braun-Fahrländer suggested that there may be factors associated with occupations related to agriculture, and thus parents who farm, may pass on a reduced risk to their children for producing specific IgE antibodies to aeroallergens, thereby preventing the development of clinical symptoms of allergic rhinitis.[14] Therefore, we speculate that Tochigi's higher farming population might account for this discrepancy.

The prevalence of allergic rhinoconjunctivitis was higher in older children, and there were no clear gender differences noted. In all age groups, the peak prevalences were observed during March and April, a period that coincides with the release of Japanese cedar pollen, which is one of the most common spring pollen antigens in Japan. Therefore, it is highly likely that the main pediatric seasonal pollen allergy that is seen in Tokyo is due to the Japanese cedar tree. When the monthly prevalence of allergic rhinoconjunctivitis was examined, it was found to be similar to the high prevalence that is seen in older children (data not shown). As seasonal rhinoconjunctivitis is a strong indicator of IgE-mediated allergy in children, our study results suggest that older children have a higher prevalence of IgE-mediated allergy than younger children.

Interestingly, the highest pollen counts during the past two decades were recorded in Tokyo during the same time when the present 10-year-old children were in their first year of life. A Swedish study that examined sensitization found that children born during a year of exceptionally high birch pollen counts had a higher prevalence of birch pollen sensitivity at ages 4 to 5.[15] In contrast, Burr showed an inverse association between grass pollen counts and the prevalence of allergic rhinitis symptoms.[16] However, they analyzed grass pollen counts in European countries, Australia, and Kuwait, and thus, their results might not be applicable to the present study in Japan.

In the current study, parents of elementary schoolchildren under the age of 11 along with a few elementary schoolchildren who were 12 years old completed the questionnaires. All of the junior high school children completed the questionnaires by themselves, which included some 12-year-old junior high school students. When reporting symptoms, answers provided by the parents and the children often differed, with the children appearing to be more valid reporters than their parents. However, the respondent differences could be ignored in the present study, as the age differences over the 12-month examination period for the three diseases did not significantly change for those who were 12 years old. Nevertheless the prevalence of exercise-induced wheezing and sleep disturbance caused by eczema more increased after the age of 11. While it is feasible that the differences were mainly because of the respondents, these differences might actually mean that some parents incorrectly judged the symptoms present in their children. Therefore, the actual prevalence of exercise-induced wheezing and sleep disturbance caused by eczema in the younger age groups could be higher than what was actually reported in the current re-